ABSTRACTS

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RNA Viruses: Mechanism and Inhibition of Viral RNA Synthesis

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Enzymic studies of viral RNA synthesis provide an insight into the mechanism of RNA virus reproduction and indicate an approach to the control of these organisms. This investigation has been performed with an extensively purified (500-fold) RNA-dependent RNA polymerase found in *Escherichia coli* infected with the RNA bacteriophage Qβ. With this enzyme, synthesis of RNA with properties similar to those of the template RNA is achieved. The reaction has certain unique characteristics. The requirement for template is satisfied only by Qβ RNA and certain synthetic polynucleotides. This template specificity is found to involve the initiation of RNA synthesis as measured by the formation of a 5′-nucleoside triphosphate terminus. Chain initiation occurs only with GTP, and polynucleotide synthesis takes place only when there is transcription of cytidine. The association of enzyme and nucleic acid has also been characterized by studies of the inhibition of the reaction. In contrast to the specificity of initiation, inhibition occurs with a variety of polynucleotides. These observations suggest the possible use of chemotherapeutic agents to block the action of viral RNA polymerase and, thereby, virus reproduction.

Elimination of Agranular Endoplasmic Reticulum in Regenerating Liver

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Following 70% hepatectomy of the rat, augmentation of ribonucleic acid synthesis occurs in the residual hepatocytes and, concurrently, their cytoplasmic organelles undergo lysosomal degradation. This study attempts to evaluate the relationship between nuclear preparations for mitosis and cytoplasmic alterations. Hyperplasia of the agranular endoplasmic reticulum (AER) was produced by the administration of 100 mg. phenobarbital/100 g. body weight for 3 days. Seventy per cent hepatectomy or sham operation was performed 24 hours after the last injection and liver biopsies were studied by electron microscopy and histochemically. Phenobarbital treatment induced hyperplasia of the AER and an increase in associated TPNH-cytochrome c reductase, which was unaffected by sham operation. In contrast, 70% hepatectomy eliminated some of this hyperplastic membrane system by lysosomal phagocytosis. The presence of hyperplastic membranes did not inhibit completely the mitotic response that followed 70% hepatectomy when phenobarbital-treated and -untreated rats were compared. Therefore, the stimulus imparted to the hepatocytes by 70% hepatectomy results in cytoplasmic dedifferentiation and eventual cell division despite intense stimulation of cytoplasmic organelle formation and function.

Studies on Sickle-Cell Anemia: Irreversibly Sickled Cells

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The presence of severely deformed erythrocytes (irreversibly sickled cells, or ISC's) in peripheral blood led to the original description of sickle-cell anemia (Hb SS disease); their role in pathogenesis has remained obscure. Current explanations of their existence assert that ISC's are relatively old erythrocytes that have suffered prior sequestration in areas of low O_2 tension. Old erythrocytes in Hb SS disease should, through processes of selective destruction, contain more Hb F than young erythrocytes, whereas low Hb F content presumably facilitates ISC formation during sequestration. Experiments were conducted to resolve these conflicting predictions.

Thin films of blood from Hb SS patients were stained for Hb F by the Kleihauer-Betke technique, and optical densities of the projected images of individual cells were measured with a gun-type photometer: mean values for ISC's were consistently less than half those for non-ISC's. Following ultracentrifugation of Hb SS erythrocytes, fractions taken along the packed cell columns were analyzed for proportions of alkali-resistant hemoglobin. This value increased in parallel with increasing specific gravity, then fell sharply as the proportion of ISC's rose to over 90% in the heaviest fraction. Cell content of total hemoglobin (MCH) was constant in all fractions: thus absolute amounts of Hb's F and S per cell varied reciprocally. In contrast, Hb A/cell in thalassemic erythrocytes is constant, but Hb F/cell varies markedly (D. Loukopoulos and P. Fessas).

These data, providing information on a morphologically curious cell, support the concept that cell content of total hemoglobin limits synthesis when a predetermined MCH is achieved. Syntheses of β^s and γ chains in Hb SS disease continue in individual cells until an MCH of normal magnitude is reached, regardless of relative amounts of Hb's F and S formed. Synthesis of β^s chains predominates in cells destined to become ISC's.

Quantitative Assay and Properties of Adenyl Cyclase

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Adenyl cyclase is a membrane receptor site for several hormones. These molecules regulate the synthesis and degradation of glycogen through the intermediacy of organ specific adenyl cyclases that transform hormonal messages into appropriate intracellular enzymic responses.

Previous adenyl cyclase assays have suffered from insensitivity, nonlinearity, and dependence upon coupled enzyme systems. The present method, which uses the heavy particulate fraction of rat brain, is also applicable to muscle and kidney. Six $\times 10^{-4}$ *M* C^{14} ATP was used as substrate. Labeled

3'5'-AMP (CAMP) was cleanly separated from the reaction mixture. Descending thin layer chromatography was used with two successive solvent systems on ion exchange cellulose. Cold marker facilitated retrieval of product for scintillation counting. Concomitant phosphodiesterase activity was measured using tritiated CAMP.

The assay detected levels of CAMP below 8×10^{-8} *M*. The reaction was linear for at least 4 minutes; during this time between 10^{-6} and 5×10^{-6} *M* CAMP was produced by 2 to 7 mg./ml. of brain protein. Brain adenyl cyclase was inhibited 50 per cent by 5×10^{-5} *M* methyl mercuric ion. Cysteine, however, did not improve enzymic activity when present during preparation or assay. Linearity was affected by the buoyancy of enzyme particles. Salt concentrations greater than 0.01 *M* caused flocculation with resulting activity loss. Phosphodiesterase activity was present in the heavy particle preparations and was not removed by washing. Even in the presence of aminophylline, between 15 and 30% of product was destroyed.

The described assay provides direct *in vitro* quantitative measurement of adenyl cyclase. The rat brain enzyme contains one or more sulfhydryl groups that are available for reaction with organic mercurials. Buoyancy of the enzyme particles is dependent on ionic strength. Accurate estimation of adenyl cyclase activity requires correction for contaminating phosphodiesterase activity.

Experimental Fetal Growth Retardation

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Reduction of uteroplacental blood flow in one uterine horn of pregnant rats was achieved by Wigglesworth's technique (ligation of uterine vessels at lower end of one horn on the 17th day of pregnancy). Cesarian section was performed on the 21st day. Fetal death or stunting occurred in fetuses located near the ligation. The most stunted fetus was compared with the corresponding fetus in the normal horn and a statistical analysis of 35 such pairs was carried for fetal weight and organ/fetal weight ratio of all organs. Stunted newborns had a mean weight of 2.77 g. vs. 4.76 g. for controls. The liver, lungs, and kidneys were the most stunted, and were affected more than the fetus as a whole, whereas the brain, placenta, and heart were least affected. The ratios for thymus, spleen, pancreas, and submaxillary gland were not statistically different from those of controls. These observations are, in part, comparable with those made in human newborns in maternal hypertension and "placental insufficiency." Histologically the organs differed little from those of control fetuses, except for lack of glycogen in livers of stunted fetuses. Even very stunted organs appeared to have matured normally. These experiments extend and confirm Wigglesworth's data on the weight of liver, brain, and placenta. They support the suggested relationship between uterine blood flow and fetal growth. They show an interesting discrepancy in the effect on growth and on maturation. *(Supported by Public Health Research Grant HED 00.743 from the National Institutes of Health, Bethesda, Md.)*

Clinical, Structural, and Functional Studies of Hemoglobin$_{Gun\ Hill}$, a β Chain Abnormality

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A Caucasian man and one of three daughters had compensated hemolysis and an abnormal hemoglobin. Hemoglobin$_{Gun\ Hill}$ (Hb_{GH}), 20% of the total hemoglobin, migrated like Hb C during starch gel electrophoresis at 8.6. An additional minor (2.5%) component was present anodal to the major abnormal band. The Hb_{GH} components were separated from Hb A_2 by carboxymethyl cellulose chromatography. An accelerated turnover of Hb_{GH} relative to Hb A *in vivo* was indicated by the specific activities of the hemoglobins both after i.v. administration of Fe^{59} and after *in vitro* incubation of reticulocytes with leucine-C^{14}. Although the α and β chains of Hb_{GH} were indistinguishable from those of Hb A after urea/starch gel electrophoresis, the abnormality was localized to the β chains by hybridization with Hb H. Peptide maps of aminoethylated β chains showed an abnormality in the region of peptides βTpX-XI. An alteration in the molecular size or configuration of the major component of Hb_{GH} was suggested by a low sedimentation velocity and by relative retardation during gel filtration. Oxygen equilibrium studies of the major component revealed an abnormally high O_2 affinity, absence of heme-heme interaction, and presence of a Bohr effect. The extinction coefficient at 430 mμ of the deoxy form of both abnormal components was lower than that of deoxyhemoglobin A, resembling the hemoglobins that do not undergo conformational changes during reactions with oxygen.

The Critical Site and Residues for Hemoglobin Function

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Structure-function relations in hemoglobin (Hb) may be a prototype for such relations in a whole class of (allosteric) enzymes. Much is known about all levels of structure of Hb and about the critical properties of function (heme-heme interaction, Bohr effect, low oxygen affinity) but local molecular mechanisms of function are unclear.

Human HbA shows two classes of ultraviolet difference spectra reflecting interactions of aromatic amino acids: 1) *p*H-dependent spectra. Carbon monoxide (CO) HbA shows peaks at 288½ and 281 mμ (therefore chromophore is tyrosine) with m*M* difference extinction coefficient (δ) 1.0. *p*K′ = 7.38 (3°); Δ H = 6 kcal/mole (therefore the perturbing group is histidine). Oxy-, met-, and cyanmetHb have the same spectrum. In deoxyHb the spectrum is much smaller (δ = 0.2). 2) Form-dependent spectra. Among CO-, oxy-, met-, cyanmetHb there is no protein-dependent difference spectrum. Compared to deoxyHb these forms show peaks at 291½ and 284 mμ (therefore

chromophore in tryptophan) with $\delta = 1.0$. Isolated HbA subunits (α^A, HbH, α^A and βAPCMB) lack the spectra of HbA, which therefore depend on interchain interactions. Dimeric ($\alpha\beta$) Hb (in 3 *M* KCl) retains the spectra; therefore they depend on contact between unlike chains.

There are 3 Tyr residues per α chain (B5, C7, H23*, nomenclature of Perutz) and 3 per β chain (Cl, H8*, H23*). Tryp is present at A12α and β and C3β. On the basis of the known 3-dimensional structure the *p*H-spectra are due to Tyr C7α perturbed by His FG4β and the form-spectra to Tryp C3β. In tetrameric Hb with dyad symmetry there are 2 types of α-β contact, $\alpha_1\beta_1$ and $\alpha_1\beta_2$ (Perutz). C7α and C3β both lie in $\alpha_1\beta_2$. Since the spectra persist in dimeric Hb, $\alpha_1\beta_2$ is retained and $\alpha_1\beta_1$ is broken. Since functional properties remain in the dimer and are known to depend on an interchain contact, the contact critical for function is $\alpha_1\beta_2$.

C3β can form a bridge between vinyl groups of α and β hemes thus forming a large interchain π-electron system; the β-chain (allosteric) shift (Perutz) which occurs with deoxygenation and on which function depends is parallel to the C-helices and changes the environment of C3β. Accordingly the C-helices (and neighboring FG corners) of the $\alpha_1\beta_2$ contact, and in particular residue Tryp C3β, are of critical importance in the functional properties of hemoglobin.

Perfusion of the Liver After Portacaval Shunt

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Critics of the side-to-side portal vein to vena cava anastomosis for relief of portal hypertension argue that loss of hepatic perfusion may result from drainage of arterial inflow into intrahepatic portal radicles and then retrograde out through the shunt without effective perfusion of hepatocytes. Catheterization studies have demonstrated that retrograde flow does occur and have permitted sampling of hepatic vein, retrograde portal, and arterial blood for comparative extraction studies. Cineangiography has provided a gross estimate of the volume of retrograde portal flow. This value multiplied by the arterial-portal difference of bromsulfthalein (BSP), yields the portal removal rate that may be subtracted from the total removal rate to yield a corrected hepatic vein removal rate. A corrected hepatic vein flow may then be estimated from the Fick formula (see formula below).

The removal rates (flow × arteriovenous difference) of oxygen, BSP, glucose, and ammonium were then compared in nine patients after operation. All patients had retrograde flow ranging from 440 to 1750 cc./min. Although the extraction per cent of the test substances averaged greater by the hepatic vein than by the portal route, the average removal rates were larger by the retrograde portal route. There were individual variations in the proportion of flow by both routes and their respective removal rates. In two patients the venous-arterial glucose difference was larger in retrograde portal blood. It was concluded that retrograde portal flow after side-to-side shunt provides quantitatively significant hepatic perfusion.

$$\text{Estimated hepatic vein flow} = \frac{\text{Total BSP removal rate—portal removal rate}}{(0.01) \times \text{arterial—HV difference}} \times \frac{1}{1 - \text{Hct}}$$

Electrolyte Concentrations, Potassium Flux Kinetics, and the Metabolic Dependence of Potassium Transport in Human Platelets

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Blood from normal human donors was collected in siliconized glass or in plastic vessels using acid citrated dextrose as the anticoagulant. The intracellular concentrations of sodium (39 mEq./l. platelet water), potassium (114 mEq./l. platelet water) and water (74.6% by weight) were determined by centrifugation of platelet-rich plasma and direct analysis of the packed platelets, with corrections for plasma trapped in the platelet buttons. The rate of flux of potassium across the platelet membrane was determined with radioisotopic potassium (K42) using standard kinetic technics. The flux rate was determined to be 112 mEq./l. platelet water per hour. In about one third of the preparations intracellular potassium appeared to be distributed in two compartments with different rates of exchange with K42. All intracellular potassium appeared to be exchangeable.

The metabolic dependence of potassium flux was studied by metabolic inhibition achieved by the use of cyanide, iodoacetate, ouabain, and anoxia. It was concluded that an active transport mechanism is present in the platelet membrane that transports potassium inward against a concentration gradient using ATP derived equally well from either glycolysis or respiration.

Ethylenediaminetetra-acetic acid (EDTA) was shown to inhibit glycolysis in platelets, probably through chelation of Mg. The production of lactate in the presence of EDTA virtually ceased but recommenced with the addition of Mg.

Clumping of platelets with ADP appeared not to alter their ability to pump potassium.

Further Characterization of the Pulmonary Blood-Gas Barrier

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An earlier report** presented a hypothesis on the role of water as a major determinant of the permeability characteristics of this barrier. On the basis of indicator dilution experiments, it was proposed that water in the barrier was structured as in normal pure water in hydrogen-bonded tetracoordinated clusters. Solutes such as ions, which disrupt normal water structure, are excluded from the barrier while solutes such as inert and

**Advances in Respiratory Physiology*, C. G. Caro, ed. London, Arnold, 1966.

respiratory gases, hydrocarbons, and short-chain alcohols enter the barrier either because they do not disrupt the clusters or because they enhance their formation. The hypothesis is supported by the results of subsequent studies with a number of other solutes of different types. In keeping with predictions based on viscosity effects, formamide and acetamide do not enter the barrier freely; propionamide and benzamide do. The monohydric alcohols from C-1 through C-4 also enter the barrier freely and have outflow patterns and distribution volumes almost identical to those of water. However, the alcohols from C-5 through C-8 have apparent extravascular volumes of distribution increasingly larger than the volumes of water as the chain length increases. The complete recovery of these alcohols in arterial blood (in contrast to the losses of the inert gases and CO_2) does not support a distribution into the gas phase but rather concentration in a nonaqueous barrier compartment. These experiments provide supporting *in vivo* evidence for the existence in the barrier of a significant lipid phase that has the important potential of rapid exchange with the vascular compartment.

Concepts and Patterns of Assistance to Failing Circulation

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The incidence of circulatory failure may be decreased and survival following cardiac resuscitation increased through the use of new concepts of function and interrelationships of lungs and heart and vasoactivity. The prevalence of arterial hypoxia suggests that altered gas transport in the lung may aggravate existing heart disease, inciting failure of the circulation. Observations will be reported that confirm the work of West, demonstrating that nondependent portions of lung may have near-optimal gas exchange, and alveolar-arterial gradients may be used to quantify abnormal ventilation-perfusion relationships. Analysis of gas tensions in blood of pulmonary veins and peripheral arteries demonstrates that changing body position can yield far greater increase in blood oxygen content than ventilation with high concentrations of oxygen.

Vasoconstriction is as common in hypoxic states, congestive failure, and pulmonary edema as it is in hypovolemia and shock, and may be overcome. The virtues of the concept of flow through capillaries has been documented by visualization of changes in microcirculation and determination of oxygen tension in lymph. The per cent saturation of oxygen in central venous blood, a reliable index of cardiac function, may be maintained or restored to normal through early and continued treatment with precise doses of adrenergic stimulating or blocking drugs and fluid and electrolyte adjustment.

Kinetics of Antibody Formation in Multiple Myeloma

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The relative rate of antibody synthesis as well as its molecular class have been studied in normal humans and in patients with various acquired immunologic deficiency disorders. Both groups of patients were immunized with 5×10^9 plaque-forming particles of T_2 coliphage and serial bleedings were assayed for neutralizing antibody. The 19 and 7S antibodies were distinguished by sensitivity to 2-mercaptoethanol (2ME). Most normal individuals demonstrated an exponential rise in 2ME sensitive antibody by 3 days following immunization. This increase terminated within the next 4 days, by which time the 7S antibody was detectable. The doubling time of the early 19S response was approximately 10 hours, while the early 7S response averaged 20 hours. In patients with multiple myeloma the latent period was prolonged by 24 to 48 hours and the doubling time for 19 and 7S production rates were also increased. Those patients with lower levels of IgG globulin produced quantitatively less antibody at a slower rate. The patients with normal levels of nonmyeloma IgG globulin or with IgA myeloma globulin were found to have normal kinetics of antibody synthesis.

Translational Control of Gene Expression Regulated by a Hormone

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The alkaline phosphatase activity of certain HeLa cell lines is markedly increased (5- to 20-fold) during growth in medium containing a glucocorticoid hormone such as prednisolone (Δ' hydrocortisone). The kinetics of enzyme induction and studies using inhibitors of protein synthesis show that *de novo* protein synthesis is required for prednisolone induction of alkaline phosphatase in HeLa S_3 cell cultures. However, there is neither an increased rate of alkaline phosphatase synthesis nor a hormone-mediated decrease in the catabolism of the enzyme since both chemical and immunological method show that despite a 5- to 10-fold increase of enzyme activity in induced cells the number of alkaline phosphatase molecules is about the same in induced as in uninduced cultures. Moreover, the physical and chemical properties of the induced enzyme differ from the base level alkaline phosphatase with regard to heat inactivation at 64.5°C., speed of elution from Sephadex G-200 and enzyme activity at all substrate concentrations tested. The base level and the induced enzyme, however, are similar with respect to certain other properties such as their Michaelis constants, electrophoretic mobility, and the degree of inhibition produced by various inhibitors of the enzyme. These findings suggest that prednisolone mediates a conformational change in alkaline phosphatase during its synthesis. Such alterations might lead to an increase in the number of catalytic sites or lowering of the energy level of the enzyme-substrate transition state. The induction by a glucocorticoid hormone of increased alkaline phophatase activity in HeLa cell cultures therefore may occur at the level of protein synthesis (translation) as a result of a steroid mediated alteration in the conformational state of the enzyme. Maximal enzyme induction is not attained until the base level enzyme has been replaced by enzyme synthesized in the presence of the hormone. *(Supported by Research Contract U-1296 from the Health Research Council of the City of New York.)*

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Lipid Metabolism in Aortas of Hypertensive Rats

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The composition of aortic lipids is altered in rats with renal hypertension. The concentrations of phospholipid and of free and esterified cholesterol are above normal while the concentration of triglyceride is essentially unchanged. The possibility that these changes may be related to synthetic activity in the vessel was investigated by studying the incorporation of acetate-1-^{14}C into aortic lipids *in vitro*. A relative increase in the fraction of acetate incorporated into phospholipid and cholesterol would suggest that aortic synthesis is a major factor contributing to the changes in composition. Whole thoracic aortas from hypertensive rats and from normotensive controls were incubated in the presence of acetate and then divided into a preparation of media plus intima and another of adventitia plus residual media. Lipids were extracted and fractionated into classes by thin-layer chromatography, and the radioactivity in the fractions measured. The difference between the amounts of acetate incorporated into lipid by tissues from the two groups of animals was greater in media-intima than in adventitia-media. Significant amounts of acetate were incorporated into all classes of lipids, but media-intima preparations from hypertensive rats incorporated three to eight times as much acetate into all classes of lipids as controls. The results reveal no significant shift in the pattern of incorporation of acetate into lipids by aortas of hypertensive rats, and may reflect a general increase in the metabolic activity of the tissue.

Electron Microscopy of Human Leukemia Cells in Tissue Culture

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Cell cultures originating from human acute leukemias and lymphomas were studied to find out if these cells are associated with virus particles recognizable under the electron microscope. The examined material consisted of two groups of cultures: the first originating from cases of acute leukemia in American patients, and the second from biopsies of African Burkitt's lymphoma. From the cytological point of view, most of these cultured cells resemble large undifferentiated blasts or stem cells. After several months in continuous culture, it becomes difficult to differentiate between cultures of myelocytic or lymphocytic origin. The prominence of cytoplasmic polyribosomes and the large size and striking electron density of the nucleoli were frequently considered as indicating the blast character of most of these cells. In all the observed cultured cells mitochondrial alterations were noticed consisting of mitochondrial gigantism frequently associated with the presence of conspicuous 30Å filaments, probably DNA. As far as the presence of virus particles is concerned, a clear difference seems to exist be-

tween cell cultures of American and African origin. In cultures of Burkitt's lymphoma cells and confirming the observations of Epstein (M. A. Epstein *et al., J. Exp. Med. 121*:761, 1965) characteristic herpeslike particles have been observed at various phases of their maturation. These particles resemble viruses of the herpes group and were observed almost exclusively in degenerating cells in intentionally "aged" cultures (1 week without refeeding). The significance of these particles remains obscure since: 1) their infective properties have not yet been demonstrated, 2) they were never observed in biopsy material from Burkitt's lymphoma, 3) their presence in degenerating cells is not a classical feature for that type of virus, and 4) their presence seems, in our series, restricted to cell cultures of African lymphoma origin. Similar particles were not so far observed in any of the cell lines of American origin, even when these were submitted to the same aging procedure. (*Supported in part by Health Research Council of the City of New York, Contract I-325, and Public Health Service Research Grant CA 08748 from the National Cancer Institute, Bethesda, Md.)*

Glucuronic Acid Conjugation of Steroids in Crigler-Najjar Syndrome

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The possibility that there are many hepatic glucuronyl transferase systems, each specific for a limited number of substrates, was examined in this study. Radioactively labeled steroid hormones of varied structure were infused intravenously into a 3-month-old female, MG, with congenital absence of bilirubin glucuronyl tranferase (Crigler-Najjar syndrome). For comparison, studies were also made in a 3-month-old female, JP, who had trisomy-21 but was otherwise normal. Complete urine collections were made following steroid infusions and the distribution of radioactivity among various urinary metabolites was determined. Following tetrahydrocortisone (THE) infusion, 27% of counts recovered in the urine of MG were extractable with CH_2Cl_2 after β-glucuronidase hydrolysis *vs.* 49% in JP. After cortisol, 21% of recovered counts were extractable after hydrolysis in MG *vs.* 42% in JP. Thus, there was a 50% reduction in the excretion of metabolites conjugated at the 3 position (3-glucs.) of C-21 steroids bear ing a 17α-hydroxyl group. After a loading dose of unlabeled THE, the findings were similar as with tracer doses. The excretion of etiocholanolone and androsterone glucuronosides (3-glucs., C-19 steroids) and testosterone-gluc. (17-gluc., C-19 steroid) was comparable in both infants. The excretion of tetrahydroaldosterone-gluc. (3-gluc., C-21 steroid lacking a 17α-hydroxyl group but bearing an 18 oxygen function), acid hydrolyzable aldosterone (18-gluc., C-21 steroid) and estriol-gluc. (16α-gluc., C-18 steroid) was normal in MG. The results indicate that in this patient with congenital absence of bilirubin glucuronyl transferase, glucuronic acid conjugation is not uniformly impaired at all sites of the steroid molecule or even at the 3 position of all C-21 steroids. The existence of multiple glucuronyl transferase systems with different substrate specificities is suggested.

Increased Net Synthesis of Lecithin by Phagocytic Cells Engulfing Particles

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Phagocytosis is associated with striking morphologic alterations involving membrane structures. Earlier studies of formation of macromolecules during phagocytosis have revealed only a small increase in *de novo* synthesis of membrane constituents such as phospholipids. Thus, no convincing biochemical counterpart for the morphologic changes has yet been found. The present *in vitro* study is aimed at exploring recently discovered alternative pathways of phospholipid (lecithin) synthesis as a possible source of membrane material during phagocytosis. Homogenates of rabbit leukocytes and alveolar macrophages contain enzymes that directly convert lysolecithin to lecithin. Resting intact cells, incubated in a medium containing ^{32}P lysolecithin, also incorporate ^{32}P radioactivity into cellular lecithin. Accumulation of ^{32}P lecithin proceeds linearly for at least 30 minutes. At equivalent substrate concentrations whole granulocytes incorporate considerably more lysolecithin into lecithin per mg. protein than do intact macrophages while homogenates of both cell types are more active than whole cells. However, the quantity of labeled lecithin associated with intact granulocytes after 30 minutes amounts to as much as 5% of total leukocyte lecithin. In macrophages this percentage does not exceed one. In the presence of polystyrene particles, incorporation of exogenous lysolecithin into lecithin is stimulated by 50% or more. Almost all of the newly formed lecithin remains associated with the cells; apparently little is released or exchanged with medium lipid. Since lysolecithin is a normal constituent of plasma, direct conversion of medium lysolecithin to cellular lecithin can therefore account for considerable addition of membrane phospholipid, especially by granulocytes. It is proposed that this mechanism of net lecithin synthesis provides building blocks for the increased membrane formation that accompanies phagocytosis.

Divergent Effects of Divalent Ions on Acetylcholine Release

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Acetylcholine (ACh) release at the motor nerve terminals of the rat hemidiaphragm was estimated in an *in vitro* system as the rate of appearance of miniature end-plate potentials (m.e.p.p.). Spontaneous m.e.p.p. frequency (2/sec.) is augmented by exposure of preparation to 20 m*M* K to about 120/sec. This high rate continues for more than eight hours and reflects continuing synthesis of quantal packets of ACh. When Ca ion concentration is increased above normal (2 m*M*) in the presence of 20 m*M* K a decline in m.e.p.p. frequency is observed porportional to the logarithm of the concentration. The low m.e.p.p. rate continues for the duration of exposure to high Ca and is reversible. When Ba is substituted for Ca at the high concentration a slow return in

m.e.p.p. frequency to its previous augmented level occurs. No change in m.e.p.p. rate occurs with interchange of bathing solutions between 2 and 16 m*M* Ba concentrations unless the preparation has been previously exposed to Ca-containing solutions. After Ca depletion of a preparation by prolonged (3 to 6 hr.) exposure to a chelating agent, m.e.p.p.s. are no longer observed; 2 m*M* Sr in the calcium-depleted preparation (K normal — 5 m*M*) gave a m.e.p.p. rate of 0.69 ± 0.10; and 2 m*M* Ba under similar circumstances gave a rate of 4.1 ± 1.4. In the presence of an augmenting concentration of K (20 m*M*) Ba (2 m*M*) gave a rate of 138 ± 41, and Sr of 107 ± 16 in the previously Ca-depleted preparation. The results suggest: 1) Ba and Sr can substitute for Ca in the ACh release process; 2) Ba can displace residual bound Ca; 3) these ions bind with different coefficients to unknown intermediates involved in release of ACh; and 4) Ba and Sr lack the stabilizing capacity of Ca on the membrane. They support the hypothesis that free Ca ions interact with an intermediate in the process of ACh release at the mammalian neuromuscular junction. *(Supported in part by Grant NB 07004, National Institute of Neurological Disease and Blindness, and I-502, Health Research Council of the City of New York.)*

Renal and Metabolic Response to Various Sodium Salts in Experimental Burns

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Despite recent emphasis on the use of isotonic saline in the early therapy of burns, the effect of various Na salts on renal function has not been delineated although therapy is guided by urine output. The rapid onset of metabolic acidosis and NaCl retention after injury, and the known diuretic action of alkaline Na salts suggested a comparison of 0.15 molar solutions of Na acetate, bicarbonate, chloride, lactate, phosphate, succinate, etc. and mixtures such as Ringer's lactate. After i.p. administration of 150 ml./kg. body wt. in two doses, urine was collected for 24 hours from groups of 5 to 10 mice and analyzed for Na, K, Cl, urea, osmolarity, and *p*H. *Normal* mice yielded maximal output of urine, Na and Cl after NaCl but one tenth or less of these outputs was excreted by burned mice. In contrast, *burned* mice excreted maximal amounts of urine, Na, K, and urea following Na acetate or HCO_3, and these outputs were several-fold greater than after NaCl. In *normal* mice, alkaline Na salts yielded outputs similar but slightly less than NaCl. Ringer's lactate or similar Cl solutions alone or containing dextran or THAM yielded outputs only slightly greater than NaCl. Potassium and urea excretion were maximal in *burned* mice after alkaline salt therapy and minimal after NaCl in *normal* mice. Acute survival was approximately equal with these Na salt solutions but the relatively smaller excretion after NaCl was reflected in analyses of skin and muscle 24 hours postburn; these gained up to one third more water, Na, and Cl than tissues of mice treated with alkaline Na salts. Insensible water loss in the first 24 hours postburn was greater in normal than in burned mice. The earlier excretion and greater output of urine and solutes after alkaline Na salts suggest their preferential use *initially* in therapy. Clinical trials have been in accord with these findings. *(Supported by Grant GM 08582 from the National Institutes of Health, Bethesda, Md.)*

Automated Hemodialysis in a Municipal Hospital

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In September 1965, a 14-bed hemodialysis center, supplied by an automated dialysate delivery system, commenced operation. Concentrated, acetate-buffered dialysate is diluted 34:1 with filtered, heated tap water by matched proportioning pumps. Prior to delivery through polypropylene tubing to individual patient stations, dialysate electrical conductivity, temperature, and flow rate are monitored. Each patient's station is equipped with a dialysate hemoglobin detector, flow meter, temperature sensor, negative pressure monitor, and venous return pressure sensor. Activation of any of the central or patient station high-low alarms causes audible and visible signals and interrupts dialysate flow. Sterilization of the central diluting unit, patient stations, and connecting tubing is effected by flushing hot water through the entire system. Fifteen men and five women, aged 17 to 57 years, with terminal renal failure, have been maintained as active outpatients while undergoing twice-weekly overnight hemodialysis. Renal function inadequate for life preservation was documented by endogenous creatinine clearance values of less than 2 ml. per minute, 24-hour urine outputs of less than 400 ml., and the failure of conservative medical management. Dialyses are conducted by specially instructed nurses under the supervision of on-call physicians. Seventeen patients have returned to full-time work or home responsibilities and three are partially rehabilitated. Complications of the therapeutic regimen include infection and clotting of arteriovenous cannulae, secondary and tertiary hyperparathyroidism, subclinical peripheral motor neuropathy, and abnormal carbohydrate tolerance. Persistent anemia has necessitated transfusion of 0.5 to 4.0 units of packed erythrocytes per patient per month to maintain a venous hematocrit of 25 per cent. Posttransfusion hepatitis has occurred four times. Serious psychiatric problems have not been encountered. Long-continued intermittent hemodialysis is an effective method of life prolongation and rehabilitation for selected patients with renal failure.

An Unusual Effect of Aspirin and Other Nonsteroid Anti-Inflammatory Drugs on Serum Albumin

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Almost all work on the mechanism of action of aspirin and clinically similar nonsteroid anti-inflammatory compounds has concerned mediators of inflammation, mitochondrial enzymes, and oxidative phosphorylation. The present report concerns evidence that almost all nonsteroid anti-inflammatory drugs (e.g., aspirin) interact with serum albumin in a unique manner. Thus it was found that the nonsteroid anti-inflammatory drugs had the ability to alter the tertiary configuration of serum albumin molecules so that they reacted more rapidly with 65 μM dithiobisnitrobenzoic acid at 30° C. (pH

7.4). A total of 180 compounds were studied, each at a concentration of 0.67 m*M*. These compounds were chosen at random to include commonly used drugs and commonly occurring biologicals and drugs with anti-inflammatory activity. Thirteen compounds accelerated the reaction between serum albumin and dithiobisnitrobenzoic acid. They were, in order of decreasing effect: indomethacin, oxyphenbutazone, phenylbutazone, flufenamic acid, ibufenac, salicylic acid, dichlorotolylanthranilic acid, hydroxydione, lauryl sulfuric acid, gentisic acid, mefenamic acid, aminophylline, and acetylsalicylic acid. Except for hydroxydione, these compounds are all known to be anti-inflammatory. No measurable effect was associated with the other 167 compounds tested, a group that included only one nonsteroid anti-inflammatory compound, aminopyrine. The administration of 3 g. of aspirin per day to human subjects produced a 33% increase in the reactivity of the subjects' serum albumin and provided direct evidence that the concentration of salicylate present in the serum of patients receiving therapeutic doses of aspirin is adequate to produce profound effects on this reaction. Salicylate did not reduce protein disulfide bonds, and the SH group was a necessary part of the reaction. These studies demonstrate an effect of therapeutic concentrations of nonsteroid anti-inflammatory compounds on a simple chemical reaction involving a serum protein and an aromatic disulfide, and may provide assistance in screening compounds for anti-inflammatory activity.

Infectious Hepatitis: Evidence for Two Distinctive Clinical, Epidemiological, and Immunological Patterns of the Disease

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As a result of the endemic environment, 1153 cases of infectious hepatitis with jaundice have been observed at the Willowbrook State School, Willowbrook, N.Y., during the past 12 years. Second attacks of hepatitis have occurred in 63 or 5.5% of this group. Recent studies have indicated that two immunologically distinct types of infectious hepatitis virus may be responsible for second attacks of the disease in certain patients. The two types of virus have been designated MS-1 (derived from the serum of a patient during the first attack) and MS-2 (derived from the serum of the same patient 6 months later during the second attack of hepatitis).

The distinctive characteristics of the two types of infectious hepatitis are as follows: 1) incubation period—ranges from 31 to 53 days for MS-1 as compared with 41 to 108 rays for MS-2; 2) abnormal serum transaminase activity—relatively short for MS-1 (3 to 15 days), relatively long for MS-2 (35 to 200 days); 3) thymol turbidity—significantly elevated for MS-1, frequently normal for MS-2; 4) contagion—MS-1 was highly contagious for all 6 intimate presumably susceptible contacts, MS-2 spread to only 2 of 5 presumably susceptible contacts; 5) immunity—patients who had an MS-1 infection were resistant to subsequent exposure to MS-1, patients with an MS-2 infection were later exposed to MS-1; their response was compatible with an MS-1 type of reaction.

These studies provide evidence for the presence of at least two types of infectious hepatitis.

Pacing on Demand in Atrioventricular Conduction Disturbances of the Heart

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In pacing on demand, ventricular stimulation is coupled to the preceding QRS complex and takes place only after a preset time interval following ventricular depolarization has been exceeded. Pacing on demand eliminates many of the dangers inherent in other pacemakers—arising from a competition between the intrinsic and electrical rhythm for capture of ventricular contraction—such as competitive or summated beats, repetitive ventricular contractions, and ventricular fibrillation caused by stimuli falling into the vulnerable period of the cardiac cycle. Pacing on demand has been found particularly valuable in patients who may revert to normal 1:1 conduction.

Therefore, pacing on demand was found to be the ideal mode of pacing in the coronary care unit, i.e., in patients who may come back to a normal sinus rhythm or in patients with a partial dissociation and unstable rhythm. External and implanted units have been tested. A method of checking the units out prior to implantation has been developed and is working satisfactorily.

In pacing on demand the electrode that delivers the electric impulse also monitors the electrocardiogram for the purpose of programming the pacemaker. It is, therefore, important to protect it from stray currents and leaking electric appliances. This has been achieved by special electronic circuitry. Conventional epicardial wire and uni- or bipolar catheter electrodes presently used with conventional pacemakers may be employed.

Chemical Mechanisms of Metabolic Inhibition

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The present study was initiated to explore by means of model systems the alternate chemical reactions possible between metabolic inhibitors and the functional groups normally present in enzymes. Representative metabolite analogs of amino acids, corticosteroids, carbohydrates, purines, and pyrimidines were treated at near physiological conditions in dilute solution (10^{-3} to 10^{-5} M) with compounds bearing the main functional groups normally present in proteins. These included the amino acids serine, tryosine, cysteine, and histidine. Rates of reaction between inhibitor and amino acid in the absence of enzymes were determined by organic functional group analysis, or by spectrophotometry. Some members of every group of analogs examined were found to react with the sulfhydryl group of cysteine to form stable products. Present results support the view that many inhibitors generally classed as isosteric antimetabolites may function by a mechanism of sulfhydryl-group linkage.

Metabolic Interactions Between Intracellular Compartments of Intact Cells

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Fractionation of tissue cells has led to a characterization of the metabolic profiles of each of the cell components, i.e., mitochondria, microsomes, nuclei, and cell sap. Information concerning the metabolic interactions between cell components has accumulated less rapidly. The present studies were undertaken to explore characteristics of intracellular transport systems in intact Ehrlich ascites tumor cells. Two transport systems were studied: 1) transfer of reducing equivalents from the extramitochondrial space to the mitochondrial compartment, and 2) a K^+-proton exchange between these two compartments. The experimental approach involved the use of inhibiting and stimulating agents with well localized and defined sites of action. The contribution of extramitochondrial-reduced pyridine nucleotides for mitochondrial oxidation was assessed after inhibiting Krebs cycle activity with arsenite, fluorocitrate, or fluoroacetate. The results of these studies indicate that these cells are unable to transport hydrogen from extramitochondrial-reduced pyridine nucleotides to the mitochondrial DPNH oxidase system. Thus, in the absence of an effective mitochondrial mechanism, the major pathway for oxidation of extramitochondrial-reduced pyridine nucleotides is pyruvate reduction to lactate. Other experiments provide evidence for an energy-dependent K^+-proton exchange across the mitochondrial membrane of intact K^+-depleted Ehrlich ascites tumor cells. In the presence of valinomycin, one observes a K^+-dependent O_2 uptake and proton release that is independent of the active transport of K^+ across the plasma membrane. As much as 50% of the high energy intermediates synthesized in the mitochondria can be utilized for the mitochondrial transport system. Studies of these two transport systems demonstrate the feasibility of this approach for the investigation of complex intracellular metabolic pathways in the intact cell.

Lipid Composition of Normal and Leukemic Human Leukocytes

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The lipids of human leukocytes vary with cell type and source. Earlier studies in this laboratory indicated that differences in phospholipid content between preparations of normal and abnormal leukocytes were due primarily to the predominance of a particular morphologic cell type. Significant differences in lipid composition have now been found between normal lymphocytes and polymorphonuclear leukocytes (isolated from blood with glass-bead columns), abnormal leukocytes from cases of acute and chronic leukemia, and polymorphonuclear leukocytes from human peritoneal exudates.

Lipids were extracted from suspensions of isolated leukocytes and analyzed for total lipid, phosphorus, cholesterol, and plasmalogens. Neutral lipids and phospholipids were studied by thin-layer chromatography. In general, the major phospholipids were (in order of descending concentration) lecithin, ethanolamine phosphatide, phosphatidyl serine plus phosphatidyl inositol, and sphingomyelin. The neutral lipid fraction contained primarily free cholesterol, with some triglyceride but little esterified cholesterol. Normal lymphocytes contained about half as much total lipid per cell as normal polymorphonuclear leukocytes, a similar cholesterol:phospholipid ratio, a somewhat lower proportion of ethanolamine phosphatide, and a higher proportion of lecithin. Normal mature leukocytes, when compared with immature cells of the same series, had a higher total lipid content per cell, with much more cholesterol and a higher cholesterol:phospholipid ratio. There was little difference in total lipid phosphorus per cell, but relatively less lecithin and more sphingomyelin.

These findings may reflect differences in the relative content of various intracellular organelles, as well as possible differences in the quantity and composition of the plasma membrane.

A Mechanism for the Antitumor, Antiviral, and Amebicidal Activities of Emetine

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A recent report that the amebicidal alkaloid, emetine, showed a dramatic therapeutic effect against granulomatous conditions in man stimulated this investigation of its mode of action. It will be shown in the present report that emetine is a potent, specific and selective inhibitor of protein synthesis. In emetine-treated mammalian cells, certain effects are observed that are secondary to the inhibition of protein syntheis. Host cell DNA synthesis is markedly reduced, and the replication of polio virus and vaccinia virus is prevented. Comparison of the inhibitory activity of related alkaloids allows a structural model to be formulated for the observed biological activity. The amebicidal properties of emetine, the known toxic effects of the alkaloid in man, and the effects of the drug on granulomas and mammalian cell viruses are readily explicable on the basis of the presently reported effects of emetine on protein synthesis. The present findings offer a molecular basis for the pharmacologic and therapeutic action of emetine. *(Supported by grants from the National Institutes of Health, Bethesda, Md., and The National Science Foundation, Washington, D.C.)*

Solubilization and Activation of Bilirubin Glucuronyl Transferase

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Bilirubin is eliminated by the liver after being conjugated through an acyl bond with glucuronic acid (GA). Other compounds are conjugated through an ethereal or an N-C kind of linkage. The glucuronidation of these compounds is catalyzed by glucuronyl transferase. Studies have been reported where results obtained with phenolic type of acceptors have been extrapolated to the conjugation of bilirubin. This extrapolation would be valid only if a single enzyme would be responsible for the transfer of GA to different acceptors.

In agreement with data obtained by others from *in vivo* experiments we have obtained *in vitro* evidence in favor of the plurality of this enzyme system. This information has been obtained after defining the optimum conditions for the *in vitro* assay of bilirubin glucuronidation and after solubilizing the enzyme. This has been accomplished by the treatment of liver microsomes with EDTA and deoxycholate. EDTA dialysis induces a tenfold activation of bilirubin glucuronide synthesis by liver homogenates. The *in vitro* activity thus obtained equals the hepatic capacity for clearance of bilirubin measured *in vivo* by B. Billing *et al.*

A New Pyrazinamide Diuretic: Reduction of the Kaliuretic Action of Ethacrynic Acid in Man

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An undesirable feature of ethacrynic acid (E.A.) as a diuretic is its accompanying kaliuresis. MK870 is a pyrazinamide with limited natruretic potency but capable of significantly diminishing K^+ secretion at the distal tubular exchange site in dogs. Accordingly, the two drugs were studied singly and in combination in patients with edema refractory to conventional diuretics. Modified balance studies were performed on the metabolic unit with daily weights, 24-hour urine electrolyte excretion, and plasma electrolyte measurements. Given alone, MK870 had minor effects on Na, K, and water excretion. E.A. alone exhibited natruresis and weight loss together with hyperkaliuria and consequent hypokalemic alkalosis. E. A. plus MK870 produced augmented natruresis and weight loss and, in addition, a blunted kaliuresis and prevention of or, in some cases, reduction in severity of hypokalemic alkalosis. In conclusion, it is suggested that E. A. (100 mg. o.d.) plus MK870 (20 mg.) is a useful combination in patients with refractory edema resulting in augmented Na excretion and reduction in the hypokalemia seen with E. A. alone.

Replication of Gross Leukemia Virus in Thymic Cultures with Induction of Cellular Neoplastic Transformation

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Gross leukemia virus (GLV) is the inducive agent of spontaneous leukemia in AkR mice. To develop a system for the study of its replication *in vitro,* cultures of embryonal rat thymus were infected with GLV grown for long intervals and periodically explored for infectious virus particles. Bioassays in susceptible animals were positive up to 100%, and electron micrographs of cultures (L. Berwick) confirmed the presence of abundant virus particles. Examination of stained coverslips, periodically removed, indicated morphological transformation of the thymic cells. Isotransplantation of these cultures resulted in tumors at the site of injection with the histological pattern of reticulum cell sarcomas. These tumors are serially transplantable and continue to carry the initial GLV as demonstrated by electron microscopy. Long-term replication of GLV in thymic cultures is important for establishing a constant source of virus, as well as for the study of virus-cell interrelationship. Neoplastic transformation of cells *in vitro* with GLV reveals a previously unknown potential of this virus and provides the system to study these events. *(Supported by Grant CA-06215-06 from the National Cancer Institute, Bethesda, Md.)*

Association of Altered Phospholipid Composition of Erythrocytes with Hereditary Nonsperocytic Hemolytic Disease

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This investigation was concerned with a family in which a fairly well compensated hemolytic disorder (mild jaundice, splenomegaly, reticulocytosis 6 to 15%) was documented in six members and was presumed to be present in eight others. Consistent changes in the lipids of RBC in hemolytic disorders have not been reported, except in acanthocytosis, in which mild hemolysis may occur. The individual phospholipids of RBC were determined by quantitative thin-layer chromatography. The proportion of lecithin in the RBC of affected members of this family was increased (34.1 to 38.4%) and

was normal (29.7 ± 1.6%) in the cells of unaffected relatives. The alteration appeared to be an absolute elevation in lecithin content, rather than a decrease in other phospholipids. The difference between the proportion of lecithin in RBC of affected family members and of normal subjects was highly significant, $p < 0.001$. RBC from other patients with hereditary hemolytic disorders and comparable levels of reticulocytosis had normal phospholipid compositions. Stained peripheral blood smears revealed occasional target cells and slight anisocytosis, poikilocytosis, and polychromatophilia. Osmotic fragility was reduced and the increase in fragility after 24 hours of incubation was less than that observed with normal cells. Autohemolysis after 48 hours was increased slightly and was corrected to nearly normal by addition of glucose. Utilization of glucose and production of lactate by the RBC were increased, the activities of 15 enzymes of the RBC were normal or elevated, and the ATP content was normal. Heinz bodies were not seen, even after prolonged incubation, and an abnormal hemoglobin could not be demonstrated. RBC lifespan in the propositus was reduced ($Cr^{51}Tl/2 = 10.6$ days), but compatible normal cells survived normally ($T1/2 = 28$ day). A causal relationship between the altered phospholipid composition and the hemolytic disorder has not been established, but both appeared to be inherited as autosomal dominant characteristics.

Growth-Related Modulation

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Clones of human heteroploid cells differing in colonial morphology provide simple systems to study factors regulating the shape and orientation of and contact between cells growing *in vitro*. The sequence of morphological changes during colonial growth, in uniform environmental conditions, of five clones derived from a HeLa-S3 strain, was studied with quantitative morphologic methods. The nature of the changes and the time at which they occur are characteristic, heritable features of each clone. A detailed analysis of the change from diffuse to compact colonies during colonial growth of one of the clones will be presented. The term growth-related modulation is introduced to describe heritable, reversible changes in cell morphology that are associated with definite stages of colonial growth. Growth-related modulation will be analyzed in terms of: 1) recovery from trauma incident to plating, 2) changes secondary to cell-cell interactions, 3) modification of medium induced by cells, 4) modification of substratum induced by cells, and 5) random events. The relevance of the concept of growth-related modulation as an analytical tool in the study of cell differentiation *in vitro* will be discussed.

Sodium Taurolithocholate: Effect on Bile Acid Excretion and Liver Function

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Since lithocholic acid is a normal fecal bile acid, is occasionally detectable in conjugated forms in bile, and causes a local inflammatory reaction, the effects of parenteral admission of the taurine conjugate on bile acid excretion and liver function were determined in hamsters and rats. Sodium taurocholate, taurochenodeoxycholate and/or taurolithocholate were given by intravenous infusion (0.1 to 1.6 μmole/min.) or injection (13 μmoles) to animals and bile collected quantitatively from a polyethylene cannula inserted in the common duct during pentobarbital anesthesia. Total bile acid excretion was estimated enzymatically (steroid dehydrogenase) and taurolithocholate-C^{14} by radioisotopic and chromatographic techniques. Bilirubin, glutamic-pyruvic transaminase, and 5′-nucleotidase were estimated in serum obtained from cannulated and noncannulated animals at appropriate intervals. Administration of sodium taurolithocholate alone caused a rapid fall in bile acid excretion followed by elevated serum enzyme levels, hyperbilirubinemia, and bilirubinuria. These effects could be prevented by simultaneous administration of either sodium taurocholate or taurochenodeoxycholate. These bile acid salts were found to enhance the rate of excretion and recovery of sodium taurolithocholate in bile. Prevention of the deleterious effects of sodium taurolithocholate is attributable to the solubilizing properties of micellar solutions of the other bile acids.

The Reaction of the Conjugated Nitro-Olefins with Protein

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The conjugated nitro-olefins may be found in exhaust products of the internal combustion engine. These compounds can provoke delayed hypersensitivity responses in the guinea pig. This immunologic activity has been correlated with the ability of the nitro-olefins to form addition products such as thio-ethers with l-cystein.

Studies of the interaction of the conjugated nitro-olefins were carried out by incubation at *p*H 7.2 of a representative nitro-olefin, 2-nitro-2-butene, with solutions of human serum albumin, human gamma globulin (Cohn FII), and egg-white lysozyme. Some denaturation of the gamma globulin was noted. Both the lysozyme and albumin,

when obtained free of the unreacted nitro-olefin, had a yellow color. Increases in the optical density at 260 to 265 mμ of solutions of both these proteins were noted. This is the region of maximal absorption of 2-nitro-2-butene. In addition, olefinated serum albumin solutions exhibited a new absorption peak at 305 mμ. The electrophoretic mobilities of the olefinated and native albumins were compared on starch gel and cellulose acetate supporting media. The olefinated albumin showed a more anodal migration. Similar studies of olefinated egg-white lysozyme indicated the presence of two bands, compared with the single band of native lysozyme. Assays for enzymatic activity of the olefinated and native egg-white lysozyme indicated a loss of ability of this enzyme to lyse the microorganism *Micrococcus leisodeikticus* after reaction with the olefin. Similar enzymatic loss was noted when human tear lysozyme was olefinated.

These studies, indicating a reaction of the nitro-olefin with protein, are consistent with their immunologic activity. They also indicate that this reactivity may cause inactivation of an enzyme involved in resistance to infection.

Fibroblast Renewal Associated with Epithelial Renewal in the Rabbit Gallbladder and Colon

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As a result of combined physiological and electron microscopic studies of fluid transport in the gallbladder and colon, it has been shown that maximal absorptive activity occurs at the crests of the mucosal folds in the gallbladder and at the free surface of the colonic mucosa (*J. Cell Biol., 27*:50A, 1965; *30*:237, 1966).

Related *in vivo* and *in vitro* studies of gallbladder mucosa labelled with ^{3}H-thymidine followed by autoradiography have demonstrated that epithelial cell replication is localized in particular sites even though the gallbladder epithelium consists of a single cell type. These sites, the valleys between mucosal folds, can now be considered analogous to the bottoms of the crypts of the intestinal mucosa, and they are, therefore, complementary to the sites of maximal absorptive function.

Electron microscopic studies have shown an adepithelial fibroblast sheath that is multilayered in the germinative zones but that forms an incomplete sheath subtending the absorptive zones of the epithelium.

The autoradiographic studies show that division of the cells of this fibroblast layer occurs during the same experimental period during which epithelial cell division is seen following a pulse of ^{3}H-thymidine. There is also incomplete evidence that these fibroblasts migrate in association with the epithelial cells. This suggests that a specialized fibroblast population may play a vital role in the differentiation and migration of the epithelial cells and basement membrane.

Ventricular Diastolic Compliance in Human Subjects

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The distensibility and elasticity of the left ventricle in diastole is of significance for physiologic studies, especially where pressure is used as an estimate of ventricular volume and when various hemodynamic interventions are imposed. Previous studies have been performed only in animals. In subjects with mitral stenosis whose natural disease acts as an indwelling orifice flowmeter, it is possible by measuring simultaneously cardiac output, ventricular volume (thermodilution method) and left atrial and ventricular pressures, to determine at 0.04-second intervals of diastole the ventricular inflow, volume (V), and radius (r), and to construct curves of $\Delta P/\Delta V$, total pressure/volume (P/V), tension-length (T/r) and elasticity (T vs. $\Delta r/r$). These curves in the resting condition were compared with changes after isoproterenol (0.8-4.0 μg./min. i.v.), l-norepinephrine (12-22 μg./min. i.v.), supine leg exercise, lanatoside C (0.6-1.0 mg. i.v.), and electrically induced tachycardia (rates 60 to 158/min.) in 33 subjects. One subject was studied during coupled extrasystolic potentiation.

The curves of distensibility, P/V, were similar to those of T/r, and were generally linear or slightly curvilinear, without evidence of plasticity. There was no significant change in shape of these curves, or of the slope of $\Delta P/\Delta V$, indicating unchanged distensibility or stiffness ($\Delta T/\Delta r$), regardless of left- or rightward shifts of the absolute P/V curve. Elasticity was linear until after onset of ventricular systole, at extensions up to 16% of initial length. There was no effect on the slope of T vs. $\Delta r/r$ (elastic modulus). The data indicate that catecholamines, digitalis, exercise, induced tachycardia, and coupled pacing shift P/V and T/r curves primarily by their effects on systolic functions and end-systolic volume "set," without affecting diastolic mechanical properties.

Monoamine Oxidase Activity of Brain Tissue in Experimental Puppies

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Monoamine oxidase (MAO) content of specific areas of brain tissue was determined in a series of control puppies and those subjected to 3 hours of hypoxia and hypercapnia. Our modification of the radioisotopic assay procedure of Otsuka and Kobayashi (*Biochem. Pharmacol. 13*:995, 1964) was used. Similar results were obtained in the control and hypercapnic puppies. However, in the hypoxic animals the combined pitui-

tary and hypothalamic areas showed a definite elevation of MAO titers and the cerebellum a definite diminution of enzyme activity. Other areas studied, i.e., pons, stem, thalamus, and cerebral cortex showed no changes. See accompanying table.

MEAN MAO UNITS/G. BRAIN TISSUE

						Cortex		
	*P and H**	*Thalamus*	*Stem*	*Pons*	*Cerebellum*	*Anterior*	*Central*	*Posterior*
Control	95,485	96,382	83,831	88,166	77,715	86,281	93,727	97,046
Hypercapnia	95,300	90,904	89,625	92,597	81,226	92,203	91,884	100,282
Hypoxia	121,445	93,980	79,305	88,168	56,034	87,501	85,894	85,702

*Pituitary and hypothalamus.

An Immunochemical Study of the Le^a and Le^b Blood Group Antigens

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Anti-Le^a and anti-Le^b isoagglutinins are weak nonprecipitating antisera that usually require fresh, enzyme-treated cells to yield reproducible results. The poor and variable reactivity of anti-Le^b sera with A_1 and B cells has precluded general agreement on the specificity of these sera, and the inhibition of many anti-Le^b sera by H substances has led some investigators to question the existence of an anti-Le^b specificity distinct from anti-H. Antisera to Le^a and Le^b substances isolated from ovarian cysts were prepared in goats. The precipitation of Le^a substance by its antiserum was inhibited by oligosaccharides of known Le^a specificity, but not by oligosaccharides possessing A, B, H, or Le^b specificity. The anti-Le^b precipitin reaction was inhibited by an oligosaccharide possessing Le^b activity, but not by oligosaccharides with A, B, H, or Le^a activity. After absorption with Le(a–b–) cells the anti-Le^a serum agglutinated Le(a+b–) cells strongly, and Le(a–b+) cells weakly. The absorbed anti-Le^b serum reacted strongly with Le(a–b+) cells and to a lesser degree with Le(a+b–) cells. A sample of 312 bloods obtained from Negro, Puerto Rican, and Caucasian donors were typed with the goat antisera. Nineteen per cent were Le(a+b–), 72% were Le(a–b+), and 9% were Le (a–b–). These results were independent of ABO type. The production of precipitating Lewis antisera of defined specificity by immunization of goats with purified ovarian cyst mucopolysaccharides has made available strong reagents for blood typing. Moreover, it affords an opportunity to detect and measure these antigens in mucous secretions, and on the surface of erythrocytes and other cells.

Purification and Properties of the 5′-Nucleotidase of Escherichia coli

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A cobalt-stimulated 5′-nucleotidase has been purified some 2,000-fold from *Escherichia coli* by means of the osmotic shock treatment of Neu and Heppel followed by DEAE and hydroxylapatite column chromatography. The enzyme is highly pure as indicated by disc gel electrophoresis, molecular sieve chromatography and sedimentation properties. The molecular weight calculated from sucrose-density gradient centrifugation and sephadex chromatography is about 53,000. The enzyme is stimulated about 100-fold by 5×10^{-3} *M* Co++ at *p*H 6 and about 200-fold when Co++ and Ca++ are present. The cobalt requirement can be partially replaced by Mn++. The enzyme in dilute solution is unstable in the absence of albumin. The *p*H optimum is 6.0 with essentially no activity at *p*H 4 and 8. The enzyme is inhibited by 0.1 *M* Zn++, but it is not inhibted by up to 0.3 *M* phosphate. It is active against all 5′ ribo- and deoxyribonucleotides with optimal activity against 5′ AMP. The enzyme is inactive against 2′, 3′, or cyclic phosphates. It will cleave the 5′ phosphate of di- and triphosphates only in the presence of cobalt because of the metal chelate formed by the nucleotide and cobalt. It has not been possible to separate the 5′-nucleotidase and UDPG-pyrophosphatase activity of the enzyme, and ratios of specific activity remain constant through purification. The enzyme is shown to be located between the cell wall and the periplasmic membrane. Within the cell a protein inhibitor has been found that inhibits the activity against AMP, ATP, and UDPG. The role of the 5′ nucleotidase and its inhibitor remain unclarified. Both are constitutive. A similar 5′-nucleotidase activity and inhibitor activity has been found in *Salmonella typhimurium, Proteus mirabilis,* and *Klebsiella aerobacter.*

A New Form of Congenital Adrenal Hyperplasia

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A new form of adrenal hyperplasia in a 12-year-old boy is described. The unique features of this syndrome are: 1) classical signs of hyperaldosteronism, i.e., benign hypertension, hypokalemic alkalosis, low plasma renin, expanded plasma volume, and hyperaldosteronism unresponsive to sodium restriction or sodium administration; 2) low normal plasma levels of cortisol, corticosterone but elevated plasma aldosterone levels; 3) elevated plasma ACTH levels; 4) sluggish response to ACTH administration of the baseline low normal urinary free cortisols, 17-hydroxycorticoids, and 17-ketosteroids, pregnanetriols and pregnanediols; 5) normal response of plasma testosterone to chorionic gondatrophin administration; 6) decrease of aldosterone production to low levels and marked fall in elevated blood pressure following treatment with glucocorticoids; 7) after 4 months of continuous therapy with prednisone, blood pressure has remained normal and the aldosterone response to sodium restriction and sodium administration returned to normal. Extensive metabolic studies and steroidal determina-

tions utilizing the double isotope dilution derivative technique indicate that an overproduction of an ACTH-dependent adrenal steroid (or steroids) was responsible for the syndrome. On the basis of steroidal data obtained, a partial 17-hydroxylase defect in the adrenal and not the gonad appears to explain the syndrome best. This form of hypertension is noteworthy because it is alleviated by medical treatment and may be misdiagnosed as primary hyperaldosteronism. See accompanying table.

BASELINE VALUES

Urine aldosterone	*Plasma aldosterone*	*Plasma ACTH*
17-26 μg./d	24 mμg. %	1.5 mUnit %
(normal 14)	(normal 3-15)	(normal 0.3-0.7)

Measurement of Exchangeable Intracellular Thyroxine in Man

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The differential exit of exchangeable intracellular thyroxine (T4) and I-125 albumin from plasma during the first 4 hours following their simultaneous administration can be used to calculate the size and kinetic characteristics of the total intracellular (= extra-albumin) compartment of exchangeable T4. The analysis is based on the demonstration that the kinetics of I-125 albumin distribution are representative of those of the other T4-binding plasma proteins and that I-131 T4 does not mix more rapidly in the total plasma protein compartment than does I-125 albumin itself. A closed two-compartmental model is used to calculate the appropriate fractional transfer contestants and the permeability characteristics of the hypothetical membrane separating the two compartments. In normal subjects, approximately 37% of tracer thyroxine is ultimately distributed to the intracellular compartment. The unidirectional cellular clearance is approximately 43 ml./min., far in excess of the normal metabolic clearance. Partition of T4 between cellular and extracellular compartments depends on the relative strength of binding by plasma proteins and by as yet unspecified intracellular factors. In patients with thyrotoxicosis, congenital decrease of thyroxine-binding globulin, and subjects receiving infusions of dipenylhydantoin, an increased fraction of administered I-131 T4 is distributed to the cellular compartment. Conversely, in patients with hepatic cirrhosis there is diminished cellular uptake of I-131 T4. Direct external hepatic measurements and liver biopsies indicate that the liver is an important component of the intracellular space. Radioautographic and chromatographic studies in experimental animals confirm the intracellular localization of I-125 T-4 shortly after injection and indicate that the rapid reversible interchange of T4 between cellular and extracellular compartments is not accompanied by a net metabolic transformation of the tracer. These findings emphasize the importance of intracellular factors in the regulation of the circulating thyroxine concentration.

Infrared Spectroscopic Examination of Cornea and Lens of the Human Eye by the Attenuated Total Reflectance Technique

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Recent studies on aorta and other tissues (*Bull. N. Y. Acad. Med. 42*:415, 1966) have shown the value of the attenuated total reflectance technique (ATR) in demonstrating biochemical lesions. The methodology has been extended to the study of cornea and lens autopsy specimens of wet, normal and diseased tissues. A thallium bromide-iodide trapezoidal plate 50 × 20 × 1 mm. with ends beveled at 45° was used in contact with tissue on one side only in a Wilks ATR unit in a Perkin-Elmer model 521 with a 621 Nernst glower. A wire screen was used in the reference beam. Many absorption bands are common to most types of biological tissue, although direct *transmission* IR methods on wet cornea do not even allow these bands to appear. In a comparison of normal lens nucleus with cataractous lens the following differences are found: band shoulder around 3100 $cm.^{-1}$ compared to shoulder missing; band around 1740 $cm.^{-1}$ compared to band missing; in normal lens 1450 intensity is greater than that of 1385, whereas in cataractous 1450 intensity is less than that of 1385. Intensities of bands of 3000 to 2800 range are quite different for normal and cataractous. Band shapes differ from 1550 to 1500. In normal cornea 2950 intensity is greater than 2850; in opaque cornea 2850 has the greater intensity. The 1630 and 1545 bands are much deeper in the normal than in the opaque specimen. Around 1400 and 1100 to 1000 band shapes are different. At 960 no band appears in normal cornea, but a weak band is present in the opaque specimen. The ATR method obviously demonstrates gross chemical changes, and should be helpful in elucidating pathological eye biochemistry.

Metabolic Alteration in the Aging Erythrocyte

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Metabolic alterations occur in the red cells that ultimately lead to disapperance from the circulation. In order to evaluate the mechanism of red cell aging, rabbit erythrocytes (free of leukocytes and platelets) were fractionated in groups of progressively mean age by ultracentrifugation in high density discontinuous gradient of bovine serum albumin. The efficiency of fractionation was evaluated by dating the cells with Fe^{59} and glycine-2-C^{14} pulse labeling.

When the red cells were nitrited and incubated with glucose, with and without methylene blue, a sharp decrease was observed in the capacity to reduce methemoglobin (mthgb.) in the older cells. The NADH pathway in the older cells was intact, both in the level of NADH-generating enzymes (3-phosphoglyceraldehyde dehydrogenase and lactic dehydrogenase) and of the transporting enzyme ("diaphorase"). In fact, the capacity to reduce mthgb. was not

altered when lactic acid or glyceraldehyde were used as a substrate instead of glucose. The NADPH-dependent pathway showed a marked decrease in the older red cells. This was found due to a reduction in one of the NADPH-generating enzymes (glucose-6-phosphate dehydrogenase); neither 6-phosphogluconate dehydrogenase nor mthgb. reductase activity were found decreased in the older red cells. As the defect in mthgb. reduction is observed even in absence of methylene blue and thus of stimulus to NADPH production through the pentose pathway, the primary defect must lie in glucose utilization. The activity of hexokinase in the older red cells was found to be one third of the youngest red cells. Hexokinase, the lowest activity enzyme in the red cells, is probably a rate limiting step in glucose utilization. A decrease in hexokinase in the aging erythrocyte explains the diminished utilization of glucose through glycolysis as well as the pentose shunt and it might be one of the important factors in red cell senescence and death.

Autoimmune Response to Thermal Injury

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The inability of the severely burned mammalian host to resist invasion by frequent pathogens (*Pseudomonas pyocyanea,* e.g.) and suggestions of the release of altered tissue constituents from the site of injury, raise the possibility that severe burns may induce alterations in host immunological function. This possibility has been explored by a study of serum and thoracic duct lymph obtained from burned Fisher rats at various times after injury. Rats received 15% body surface area full-thickness skin burns by timed contact with a metal plate (250° C.). Serial thoracic duct lymph samples were studied for: 1) total lymphocyte output, and 2) presence of autoantibodies directed against normal Fisher rat erythrocytes. Sera obtained during the same periods from burned animals, as well as sera and thoracic duct lymph samples obtained from normal animals were studied in similar fashion. Results of studies in 60 consecutively burned rats and in 12 normal controls indicate that: 1) there is a sharp decline in the total lymphocyte output in burned animals within the first 24 hours after injury; this effect is still in evidence as late as 56 days after injury; 2) burn lymph contains a 7S antibody capable of specifically agglutinating normal Fisher erythrocytes; 3) this autoantibody is absent in burn sera or in normal lymph. The results indicate that severe burns may induce in Fisher rats a state of autosensitization to their own erythrocytes and a fall in the total circulating lymphocyte count. These observations may have a possible bearing upon the course and prognosis of severe thermal injury. *(Supported by Contract NONR-4503, Office of Naval Research, Washington, D.C.; by Public Health Research Grant GM 12748-01 from the National Institutes of Health, Bethesda, Md.; and in part by a grant from the John A. Hartford Foundation, Inc., New York, N. Y.)*

Biological Alterations in Human Recipient Reactivity to Skin Allografts

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Leukocyte surface antigens detected by hyperimmune sera obtained from multiparous women and polytransfused patients have been shown to be active as transplantation antigens. In addition, mechanical disruption and differential centrifugation of blood leucocytes has yielded cell-free cytoplasmic particulate components capable of inducing allograft sensitivity in man.

In an attempt to facilitate the survival of human skin allografts, 31 healthy subjects selected by leukocyte grouping techniques were pretreated with cytoplasmic leukocyte extracts obtained from the prospective graft donors. The preparations were injected by the intradermal, subcutaneous, and intramuscular routes, in doses varying from 4 to 500 times the concentration capable of inducing graft sensitivity. Two weeks later, the recipients were tested with skin allografts obtained from the same donors. The mean survival time of skin allografts in this group of individuals was 15.6 days. The longest graft survival (graft still intact at 68 days) was noted in a donor-recipient combination in whom no major leukocyte group incompatibilities were detectable. These data are in contrast with the mean survival time of 10.1 days previously observed in 71 normal, untreated, randomly selected subjects.

The combined use of leukocyte grouping techniques and pretreatment with cytoplasmic extracts of leukocytes may offer an approach to the conditioning of human recipients to skin allografts. Further progress in this direction awaits the availability of a soluble, biologically active preparation of transplantation antigens suitable for intravenous use. *(Supported by a grant from the John A. Hartford Foundation, Inc., New York, N. Y.)*

Single Neurone Activity in Hippocampus and Amygdala of Man

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Previous exploration of cortical epileptiform spike foci of man with one and two microelectrodes have revealed that more than one pattern of intracortical slow potential distribution and of single neurone discharge underlie the generation of the epileptiform spike recorded at the pial surface, these intracortical activities resembling those observed in cortical evoked potentials in the experimental animal. Neu-

ronal activity patterns implied by Jackson's classic hypothesis of "excessively discharging gray matter" were not observed (M. Rayport and H. J. Waller, 1961-66). These findings being consistent with the possibility that a preponderance of spike foci in cortex exposed at craniotomy are the result of projected activity from deep gray matter, single neurone recording was undertaken in the hippocampal formation and amygdala in patients with intractable epilepsy.

During stereotaxic-EEG exploration, tungsten microelectrodes with tip diameters of 1-3 micra have been oriented according to the anatomical criteria of the Talairach school and advanced with a micromanipulator mounted on the stereotaxic instrument. Single neurone recordings have been made during spontaneous electrical activity with the subject awake or in light sleep; during attention and higher mental performance (naming, reading); during olfactory and electrical neocortical and rhinencephalic stimulation and electrical after-discharge; during interictal epileptiform EEG phenomena; during spontaneous and bemegride-induced seizures.

Abnormal Composition of the Lipophilic Components of the Brain Gangliosides in Infantile Amaurotic Idiocy (Tay-Sachs Disease)

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Monosialoganglioside lacking a galactose residue accumulates in the degenerating brain of children with Tay-Sachs disease. This study provides new clues to the chemical aberration leading to the degenerative process. Fatty acids of gangliosides were identified by gas liquid chromatography, sphingosine by examination of permanganate-periodate oxidation products. Fatty acids and sphingosine of gangliosides from diseased brain were compared with those of the various brains of normal humans and rates of varius ages. Tay-Sachs gangliosides had an unusually high content of stearic acid and sphingosine almost exclusively of the 18-carbon variety. Gangliosides from the brain of normal children had a lower content of stearic acid and almost equal quantities of 18- and 20-carbon sphingosine. The pattern found in the gangliosides of the diseased brain was that found in the gangliosides of the brain of the normal human and normal rat immediately after birth. These results indicate that a failure of chemical development may underlie the massive accumulation of monosialoganglioside that accompanies degeneration of the brain in Tay-Sachs disease.

Evidence for Evolutionary Changes in Fat Cell Physiology

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The rat's fat cell performs four functions, each of which is hormonally regulated: conversion of glucose to fatty acids (FA) (lipogenesis); uptake of extracellular triglyceride-FA; storage of FA as intracellular triglyceride (TG); and mobilization of stored TG. This laboratory has now examined *in vitro* patterns of glucose metabolism and hormonal responsiveness of adipose tissue (AT) of 6 mammalian species, and other laboratories AT of 3 additional mammalian, 4 avian, and 3 insect species. Results of all these studies show: 1) vertebrate fat cells utilize glucose as the major extracellular carbohydrate, but insect fat cells use trehalose instead; 2) among vertebrate AT, conversion of glucose to FA is a major pathway in rodents of Muridae and Caviidae families but not in rodents of Cricetidae family, in the lagomorph order, or in the bird class; 3) insulin stimulates glucose transport into fat cells of Muridae and Caviidae but not of Cricetidae or lagomorph mammals, or of birds; 4) stored TG is mobilized by insect AT as diglyceride but by vertebrate AT as FFA; and 5) in rodent and lagomorph mammals, pituitary peptides stimulate FFA mobilization; in all other mammal and bird species tested, catechol amines are the principal stimuli of this process. These data indicate major alterations in the lipogenic and mobilization functions of the fat cell during invertebrate and vertebrate evolution.

Metabolism of Hyaluronateprotein in the Wrist Joint of Calves

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Hyaluronateprotein (HP) is a large polymer that is responsible for the viscosity of synovial fluid (SF). Since the protein moiety of HP can be labeled with I^{131}, the metabolism of HP in SF can be approached experimentally. HP, isolated from bovine SF by ultrafiltration on 0.1 μ millipore filters, contained 3% protein. Bovine HP was labeled with I^{131}; free I^{131} was removed by sequential dialysis, zone electrophoresis, and ultrafiltration of HP-I^{131}. One ml. HP-I^{131} (100 γ HP, 1 to 10 microcuries I^{131}) was injected into the wrist joint of a calf. The rate of removal of HP-I^{131} was determined in two ways: 1) by daily aspiration of SF and serial determination of specific activity $\frac{I^{131}\text{ (CPM/ml.)}}{\text{HP }(\gamma\text{/ml.})}$ of HP isolated from each sample of SF, and 2) by daily external counting of I^{131} over the joint. For 7 to 14 days determinations of I^{131} were performed on the blood, urine, and stool. When the values for specific activity of HP-I^{131} were plotted on semilog paper, a straight line

was obtained. The half-life of HP-I^{131} varied from 15 to 24 hours in 6 experiments performed in 4 different calves. Two straight lines could be extrapolated from the curve obtained when the data from external counting were plotted. During the first 4 to 6 days of the experiment the rate of removal of HP-I^{131} from the joint was similar to the half-life of HP-I^{131} in the SF. After the sixth day the rate of removal of I^{131} from the joint slowed markedly. Within 1 hour after injection of HP-I^{131} into the joint, I^{131} appeared in the blood. Within 24 hours a significant amount of I^{131} was found in the urine. Very little I^{131} appeared in the stool. Throughout the experiment the HP-I^{131} in SF remained nondialyzable; the I^{131} that appeared in the urine was dialyzable. Thus far the data obtained in the studies indicate that: 1) HP is rapidly removed from SF; 2) HP does not appear to be degraded in the SF; 3) HP is not removed from the joint as rapidly as from the SF; 4) HP is degraded either in the joint or elsewhere in the body after it is removed from the joint, and 5) some of the degradation products of HP are excreted in the urine.

Adaptive Regulation of the Biosynthesis of a Specific Cell Protein: Apoferritin

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The biosynthesis of apoferritin *in vitro* is a useful probe for studying cell differentiation, i.e., regulation of the synthesis of specific proteins. Apoferritin binds and stores iron to yield ferritin, and both forms of the protein can be crystallized or readily quantified and isolated as immune precipitates. For *in vitro* studies slices of rat liver (or spleen, duodenum, or kidney) are incubated with C^{14}-leucine, subsequently apoferritin and ferritin are isolated by precipitation with specific antiserum, and both net synthesis and incorporation of C^{14} are estimated. Doses of 1 to 9 mg. Fe/100 g. rat given 2 to 24 hours prior to sacrifice enhance incorporation of C^{14}-leucine into apoferritin by liver slices *in vitro* up to tenfold, and net synthesis is observed. Actinomycin D, an inhibitor of DNA-dependent RNA synthesis, markedly inhibits this adaptive response to Fe. Moreover, Fe administration increases the subsequent incorporation of C^{14}-orotic acid, given as a 10-minute pulse to intact rats, into the rapidly labeled fraction of liver nuclear RNA. Thus iron appears to act on a genetic locus to increase apoferritin synthesis. Following actinomycin D the capacity to synthesize apoferritin decreases with a half-life of about 4 to 5 hours, and this may reflect the half-life of the corresponding messenger RNA. *(Supported by Public Health Service Research Grant 04407 from the National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.)*

Enzyme Induction in Regenerating Rat Liver

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It is well known that changes in the physiologic status of an animal (administration of hormones, starvation, etc.) results in predictable alterations in the activities of many enzymes in the liver and other tissues. Hepatomas, however, have been shown to be largely unable to respond to these stimuli with the expected changes in enzyme activity. Some workers feel that this absence of normal enzymatic regulatory mechanisms may represent a fundamental biochemical lesion in malignant neoplasms. The activity of the hepatic enzyme tryptophan pyrrolase (TP) is increased several times within a few hours after cortisone administration. This effect of cortisone is absent or markedly reduced in hepatomas.

The induction of TP by cortisone was studied in regenerating rat liver in an attempt to determine whether a dividing but nonneoplastic population of cells would respond normally to steroid administration. At various times after 70% hepatectomy, adrenalectomized rats were given cortisone i.p. They were sacrificed 4 hours after steroid administration, and TP activity was assayed. Sham-operated adrenalectomized rats were used as controls.

The results show a marked decrease in the degree of TP induction in the regenerating liver, beginning at a time coinciding with DNA synthesis. This suggests that the findings observed in hepatomas may not be specific for neoplasms.

The Diagnosis and Therapy of Shock, Using Physiologic Correlates Quantified with a Bedside Computer

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Cardiovascular dynamics and oxygen consumption were measured in patients in shock from sepsis, hypovolemia, or pulmonary embolus, and five physiologic correlates immediately derived using an Olivetti Programma 101 bedside computer: 1) net vascular tone; 2) effective oxygen transport; 3) relative peripheral shunting; 4) pulmonary venoarterial admixture; and 5) ventricular function. Each of these was graphed as the patient's condition indicated on a nomogram for that particular function previously derived from analysis of 84 patients. This analysis showed that, compared both to normal and nonseptic shock patients, patients in septic shock have a net vascular tone that is decreased to a degree comparable to that found in severe liver disease. Determination of the effective oxygen transport and relative peripheral shunting functions has enabled discrimination of a hyperdynamic and hypodynamic group amongst the patients in septic shock. Both relative peripheral shunting and pulmonary venoarterial admixture were greater in the patients in hyperdynamic septic shock than in any other group, and correlated well with the severity of the shock. Evaluation of the ventricular function relationships showed that decreased cardiac function is a factor relatively early in the clinical course of nearly all patients in septic shock, and that a worsening ventricular function relationship is associated with a deteriorating clinical course. This myocardial failure can often be reversed by administration of an inotropic agent. Using computer determinations of these physiologic correlates and the appropriate nomograms, it is possible to follow an individual patient's clinical course and make therapeutic decisions at the bedside based on the nature and extent of the individual deviation from normal.

Oxandrolone in the Treatment of Short Stature

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Six boys and two girls, aged 4 to 18 years, were treated with oxandrolone, 0.2 mg./kg. daily during the second 6 months of an 18- to 24-month period. Measurements of height and of skeletal age were made at the beginning and at 6-month intervals, and results were evaluated in terms of increments in height age and in skeletal age. The rate of increase in height age was significantly increased in the 6 months of treatment. This analysis was made by applying the one-tailed ranking test to the difference between height age advance during treatment and that before treatment for each child individually. Similar analysis of increments in skeletal age showed no difference attributable to treatment. The average 6-monthly increment in skeletal age minus height age was calculated for each child for the 12 to 18-month period that included treatment and post-treatment follow-up. This number was subtracted from the increment in skeletal age minus height age during the 6 months preceding treatment. Analysis of these figures showed no significant effect, by either T test or ranking test. However, 2 of the 8 children showed an excess increase in skeletal maturation: one of 6 months and one of 12 months. This observation indicates that caution should be exercised when this drug is used in an effort to improve rate of growth.

Plasma Diamine Oxidase as an Index of "High Risk" Pregnancy

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The definition of "high risk" pregnancy is currently based upon clinical parameters, many of which have been shown to be inadequate guides to the outcome of a particular pregnancy. In the present study, serial plasma diamine oxidase (DAO) titers (radioassay procedure) were determined in a series of subjects with 1st and 2d trimester complications of pregnancy in an attempt to delineate further the "high risk" group. DAO titers within the normal range consistently indicated the maintenance of pregnancy into the 3d trimester. For this reason the plasma DAO assay is uniquely applicable to problems of the first trimester unlike estriol determinations, which assume prognostic significance toward the latter half of pregnancy. A falling or persistently low level of plasma DAO in early pregnancy was associated with significant fetal wastage. There were, however, some pregnancies with abnormal enzyme titers that continued to successful term deliveries. Despite these live births there is evidence to suggest that infants born of these pregnancies have an increased incidence of neurological impairment. Thus, serial plasma DAO titers appear to be of value both in the identification of the "high risk" group of pregnancies and in the selection of the infant who will require long-term comprehensive observation.

DNA, RNA, and Protein Synthesis in Human Lymphocytes Stimulated with Phytohemagglutinin (PHA)

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When PHA is added to suspensions of blood lymphocytes there occurs a prompt increase in the rate of desoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein synthesis. A period of growth commences and, during the succeeding 72 hours, a majority of the cells undergo enlargement (transformation) and some enter mitosis. In the present study, the effect of actinomycin D (5 γ/ml.) and of puromycin (10 γ/ml.) on DNA, RNA, and protein synthesis *in vitro* of PHA-stimulated normal lymphocytes was measured employing ^{3}H-thymidine, ^{3}H-uridine, and ^{3}H-leucine. Cultures were set up in triplicate employing the technique of Bach and Hirschhorn (*Science 143:* 813, 1964). Inhibitors were added at the same time as the tracers and isotope incorporation was studied over a 4-hour period. DNA synthesis in PHA cultures is minimal on day 1; however, it increases 20- and 42-fold compared to controls on days 2 and 3 respectively. On days 1, 2, and 3, PHA stimulates RNA synthesis 5-, 13-, and 15-fold, respectively, compared to controls; protein synthesis is increased 3-, 6-, and 14-fold. Actinomycin D inhibits the synthesis of RNA 100%, DNA 80%, and protein 50%, compared to untreated PHA cultures. The results are consistent with the view that actinomycin D, by completely blocking DNA-template function in RNA synthesis, inhibits formation of new RNA(s) and thus inhibits formation of new protein. This decrease of protein synthesis or the direct inhibition by puromycin leads to a decrease of enzyme(s) essential for DNA synthesis. Continuation of protein synthesis at a reduced rate following actinomycin D supports the hypothesis that a portion of the messenger templates is stable.

Effects of Infusion of Recirculated Pump-Oxygenator Blood

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Pulmonary damage of obscure etiology occurs after pump-oxygenator procedures. To study its pathogenesis, dog blood was recirculated through a sterile system containing heat and gas exchangers. Aliquots of this blood or its derived plasma or washed cells were infused intra-arterially into 10 dogs, or intravenously into 5. Portal, systemic and pulmonary, hemodynamics and pulmonary function and morphology were monitored before, during, and after infusion. Recirculated blood or plasma infusion by

either route regularly produced elevation of pulmonary vascular resistance, compliance and impaired gas exchange. Morphologically, recipients showed varying degrees of pulmonary damage characterized by hemorrhage and edema. Distinctive periarterial hemorrhage was an early or minimal finding that preceded all other changes, and was identical to that in lungs of patients dying after cardiopulmonary bypass and in dog lungs after venovenous bypass. Unrecirculated blood and washed cells failed to produce these changes. These results exclude many proposed causes of postperfusion damage and indicate that it results primarily from injury to small arteries or their vasa vasorum, mediated by a plasma alteration or factor that exerts specific effects on noncapillary pulmonary vasculature and is unaffected by passage through a capillary bed.

Biochemical Investigation of the Inherited Variation in Drug (INH) Acetylation

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Many drugs are metabolized in the body by reaction with endogenous compounds to form inactive derivatives (e. g., by acetylation or glucuronidation). We are investigating the biochemical basis for the genetically determined variation in the rate of enzymatic transfer of the acetyl group from acetyl coenzyme A to isoniazid (INH) and sulfonamides in man and the rabbit. Our investigations involve the comparison of the acetyltransferase obtained from various INH inactivator genotypes and rely upon knowledge of both the relationship between enzyme concentration and activity and the characteristic kinetic constants of the enzyme.

We have studied the kinetics of the inactivation of INH by acetyltransferase partially purified from rabbit liver (300-fold from the $10^5 \times$ g supernatant). Our initial rate data show that the reaction obeys "ping-pong" kinetics, i.e., the enzyme oscillates between free enzyme and another stable form. The latter is probably acetylated enzyme, since our product inhibition studies show that coenzyme A is a competitive inhibitor with respect to INH and a noncompetitive inhibitor with respect to acetyl coenzyme A.

An enzyme that obeys "ping-pong" kinetics cannot be saturated readily with both substrates, so that the rate of drug acetylation *in vitro* may not be proportional to enzyme concentration. Moreover, the K_m determined with one substrate, in such a system, varies with the concentration of the other substrate, and therefore any single, experimentally determined K_m is not necessarily characteristic of the enzyme. Thus, a more complete kinetic evaluation is necessary to determine the characteristics of each INH acetyltransferase before comparisons of the enzyme derived from the various inactivator genotypes can be made. Such an evaluation of our kinetic data will be presented.

The significance of these findings for studies of other reactions that proceed by complex mechanisms will be discussed with special reference to specific human pharmacological and genetic studies.

Effect of Polyene Antibiotics on Lysosomes and Artificial Lipid Structures

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Polyene antibiotics such as amphotericin B and filipin kill susceptible cells by reacting with their limiting membranes. Since fever and renal lesions are the toxic effects of polyene antibiotics, their effect on lysosomes and mitochondria was studied. Whereas filipin and etruscomycin (low molecular weight polyenes too toxic for clinical use) disrupted lysosomes from liver, spleen, and leukocytes at both acid and neutral *p*H, amphotericin B and nystatin were only disruptive for *kidney* lysosomes at acid *p*H. Polyenes were effective at concentrations above $10^{-5}M$, and none of the antibiotics proved injurious to mitochondria. Since such differences might result from differences in lipid composition of the target membranes, artificial lipid structures were prepared that resemble natural membranes in response to lytic agents (*J. Molec. Biol. 13*:253, 1965). Filipin and etruscomycin induced leak of marker molecules from phospholipid/long-chain anion spherules prepared in the absence of cholesterol. Amphotericin B and nystatin, in contrast, preferentially disrupted spherules prepared with cholesterol; the leaks induced by these varied directly with the molar ratio of cholesterol. Neither variations in sign or amount of charged components of the spherules affected polyene action, as shown by varying the molar percentages of cationic stearyl amine and anionic dietylphosphate from 5 to 20% of the total spherule lipid. It was concluded that polyene antibiotics can rupture synthetic membranes by interactions with membrane phospholipids alone; however, some polyenes (amphotericin B) require an optimum phospholipid/cholesterol ratio. The toxicity of the polyene antibiotics may be due to their interaction with membrane components common to both microorganism and host.

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